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Catabolism of raw and cooked green pepper (*Capsicum annuum*) (poly)phenolic compounds after simulated gastrointestinal digestion and fecal fermentation.

SHORT TITLE: Catabolism of pepper (poly)phenols after digestion and fecal fermentation

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Published in Journal of Functional Foods, 27, 201-213 (2016)

DOI: <http://dx.doi.org/10.1016/j.jff.2016.09.006>

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ABSTRACT

A total of 21 (poly)phenolic compounds (free and bound) were quantified in raw, olive oil fried, sunflower oil fried and griddled green pepper before and after a simulated gastrointestinal digestion. Flavonoids, particularly quercetin rhamnoside, were the main compounds. The bioaccessibility of (poly)phenolic compounds after gastrointestinal digestion was higher in cooked (>82%) than in raw (48%) samples, showing a positive effect of heat treatment on the release of (poly)phenols from the vegetal matrix. Additionally, a fecal fermentation was carried out for 24h. A time-dependent microbial metabolic activity was observed, which resulted firstly (<5h) in the hydrolysis of flavonoid glycosides and then in the formation of 3 catabolites, namely 3,4-dihydroxybenzoic acid, dihydrocaffeic acid and 3-(3'-hydroxyphenyl)propionic acid, this being by far the most abundant. Catabolic pathways for colonic microbial degradation of flavonoids and hydroxycinnamic acids have been proposed. Griddled pepper showed the highest amount of (poly)phenols both after gastrointestinal digestion and colonic fermentation.

KEYWORDS

Polyphenols; *In vitro* bioaccessibility; *In vitro* gastrointestinal digestion; Colonic catabolism; Heat treatment; Pepper

1. Introduction

Europe produces millions of tonnes of a broad range of fruits and vegetables thanks to its varied climatic and topographic conditions. Mediterranean countries such as Turkey, Spain, Italy, Greece or France are the largest producers of vegetables (Eurostat, 2015), and are also the countries characterized by the highest intake of fruits and vegetables. Plant foods are the main source of dietary antioxidants, including phenolic compounds. (Poly)phenol rich foods have been reported to exhibit a wide range of biological effects such as protective effects against cardiovascular diseases, neurodegenerative diseases and cancer, probably, but definitely not solely due to their ability to protect against oxidative damage in cells (Del Rio et al., 2013).

Phenolics are compounds with at least one aromatic ring with one or more hydroxyl groups attached and could be divided into several classes. Flavonoids are one of the main polyphenolic compounds found in vegetables. The majority of flavonoids occur naturally as glycosides rather than aglycones, like in green peppers, one of the most consumed vegetables in the Mediterranean countries such as Spain (MAGRAMA, 2015). Quercetin rhamnoside and luteolin 7-*O*-(2-apiosyl-6-malonyl) glucoside are the most abundant phenolic compounds in green peppers, accounting for around 80% of total phenolics (Juániz et al., 2016; Marin, Ferreres, Tomás-Barberán, & Gil, 2004). However, it must be taken into account that many dietary vegetables are usually eaten after different cooking methods and that (poly)phenolic compounds can be either degraded or released from plant tissue structures by thermal processes, depending on the cooking methods and their time and temperature conditions, as well as the type of vegetable (Juániz et al., 2016; Miglio, Chiavaro, Visconti, Fogliano, & Pellegrini, 2008; Palermo, Pellegrini, & Fogliano, 2014; Pellegrini et al., 2009; Ramírez-Anaya, Samaniego-Sánchez, Castañeda-Saucedo, Villalón-Mir, & de la Serrana, 2015).

After ingestion, (poly)phenols can also be modified in the gastrointestinal tract by digestive enzymes and, consequently, their bioaccessibility might be affected. The stomach reduces the particle size of food, in turn potentially enhancing the release of phenolic compounds (Scalbert, Morand, Manach, & Remesy, 2002). Additionally, glycosylation influences absorption at intestinal level, since glycosidic flavonoids are more bioaccessible than their aglyconic forms (Manach, Williamson, Morand, Scalbert, & Remesy, 2005). Deconjugation can take place in the lumen by the action of membrane-bound lactase phlorizin-hydrolase (LPH) and aglycones may then be absorbed passively through the epithelium. The epithelial cells can also hydrolyze the glycosides by the action of cytosolic β -glucosidase and consequently aglycones may be formed after absorption by the active sodium-dependent glucose transporter, SGLT-1 (Day et al., 2000; Nemeth et al., 2003). Nevertheless, the levels of flavonoids in plasma after dietary intake are low (Aura, 2008), which is probably related to their limited absorption. Absorbed compounds undergo phase II enzymatic metabolism and they can be conjugated with glucuronic acid, sulphate and methyl groups in the liver and enterohepatic recirculation may result in some recycling back to the small intestine through bile excretion, so parent compounds could not be detected in plasma (Aura, 2008; Del Rio et al., 2013). A large part of the ingested (poly)phenols could then reach the colon where they could be transformed by the local microbiota to smaller and more absorbable molecules. Gut microbiota metabolism can also modulate the health effects of dietary (poly)phenolic compounds by altering absorption, bioavailability, and biological activity, so biological effects should not be only attributed to the native compounds present in foods but also to their metabolites (Duda-Chodak, Tarko, Satora, & Sroka, 2015).

Therefore, this work aimed at (1) investigating the effect of a simulated gastrointestinal digestion on the (poly)phenolic fraction of both, raw and cooked green peppers, a vegetable

commonly consumed as crude in salads and cooked in several ways in the Mediterranean Diet, and (2) identifying and quantifying the main metabolites derived from an *in vitro* microbial colonic fermentation.

2. Material and methods

2.1 Chemical and reagents

Sweet Italian green pepper (*Capsicum annuum*), olive oil (refined and virgin olive oil blend) and sunflower oil were obtained from local stores. Selection of oils was based on their high consumption by Spanish consumers for frying.

Human saliva α -amylase (852 U/mg protein), pepsin (674 U/mg), pancreatin (4xUPS), and bile salts (for digestion) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Anhydrous dipotassium hydrogen phosphate and soluble starch were from Carlo Erba Reagents (Milan, Italy). Methanol, 99% formic acid, acetonitrile, bile salts (for fermentation), calcium chloride, (+)-arabinogalactan, tryptone, yeast extract, buffered peptone water, Dulbecco's phosphate buffer saline, casein sodium salt from bovine milk, pectin from citrus fruits, mucin from porcine stomach-type III, sodium hydrogen carbonate, potassium phosphate monobasic, magnesium sulfate monohydrate, guar gum, Tween 80, xylan from Birchwood, L-cysteine hydrochloride monohydrate and iron(II)-sulfate heptahydrate, resazurin redox indicator sodium hydroxide, ethyl acetate, and citric acid were obtained from Sigma-Aldrich (St. Louis, MO, USA). Potassium chloride and sodium chloride were obtained from Merk (Darmstadt, Germany). Pure phenolic standards for high-performance liquid chromatographic (HPLC) and tandem mass spectrometric (MS/MS) analyses of rutin, luteolin-4-glucoside, quercetin, luteolin, 5-caffeoylquinic acid (5-CQA), caffeic acid, *p*-coumaric acid, dihydrocaffeic acid, 3-(3'-hydroxyphenyl)propionic acid, and protocatechuic acid were also purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2 Samples preparation

Chopped green pepper (300 g) was fried with olive or sunflower oils (30 mL) at 115 °C for 10 minutes in a non-stick frying pan. Then, temperature was decreased to 108 °C for 5 minutes. Chopped green pepper was also submitted to a heating process at 150 °C for 10 minutes and then at 110 °C for 5 minutes in a non-stick griddle without oil addition. Then, raw and cooked green peppers were lyophilized in a freeze dryer Cryodos-80 (Telstar, Terrasa, Spain), and stored at -18°C until further analysis.

2.3 Simulated gastrointestinal digestion

A three step *in vitro* digestion model was carried out in a bioreactor according to Minekus et al. (2014) and Monente et al. (2015 b) adapted to our laboratory. Briefly, 2 g of each sample was weighted in a 100 mL vessel placed and heated in a water bath at 37 °C. The vessel was magnetically stirred and connected to a pH sensor. The three steps were carried out in absence of light. Simulated salivary, gastric and intestinal fluids (SSF, SGF and SIF) (Table 1 supplementary information) were employed for each step. First, oral digestion was performed by adding 14 mL of the stock SSF solution, 250 μ L of an α -amylase solution (1.3 mg mL⁻¹), 0.10 mL of 0.3M CaCl₂, and water up to 20 mL. The sample was shaken for 30 min at 37 °C. Second, the gastric digestion step was carried out at pH 3 with 1M HCl. It was started by adding 15 mL of SGF, 1.19 mL of a pepsin solution (1 g of pepsin in 10 mL of 0.1 M HCl), 0.01 mL of 0.3M CaCl₂ and water up to 20 mL. After 2 h incubation, the final intestinal step was carried out by adding 22 mL of SIF, 10 mL of a pancreatin solution (0.008 g mL⁻¹), 5 mL of bile salts (0.025 g mL⁻¹), 0.08 mL of 0.3M CaCl₂ and water up to 40 mL. The pH was then adjusted to 7 with 1M NaOH and the samples were incubated for 2 h. All samples were frozen and lyophilized in a freeze dryer Cryodos-80 (Telstar), and stored at -18°C until further

analysis. Each green pepper sample was digested in duplicate and then the two repetitions were mixed and homogenized.

2.4 *In vitro* fecal fermentation

The *in vitro* fecal fermentation was carried out according to Dall'Asta et al. (2012) adapted to the samples under study.

2.4.1 *In vitro* fermentation growth medium preparation

The composition for 1 L of growth medium was 2.5 g of soluble starch, 2.5 g of peptone, 2.5 g of tryptone, 2.25 g of yeast extract, 2.25 g of NaCl, 2.25 g of KCl, 1 g of pectin, 2 g of mucin, 1.5 g of casein, 1 g of arabinogalactan, 0.75 g of NaHCO₃, 0.35 g of MgSO₄H₂O, 0.5 g of guar, 0.5 g of xylan, 0.4 g of L-cysteine HCl·H₂O, 0.25 g of KH₂PO₄, 0.25 g of K₂HPO₄, 0.2 g of bile salt, 0.04 g of CaCl₂, 0.0025 g of FeSO₄·7H₂O, 0.5 mL of Tween 80, and 2 mL of resazurin solution (0.025%, w/v) as an anaerobic indicator. The growth medium was sterilized at 121°C for 15 min in glass vessels (12 mL) before sample preparation.

2.4.2 Fecal slurry

Fresh fecal samples were collected from four healthy donors who did not have previous intestinal disease, were not treated with antibiotics for the previous 3 months, and followed a polyphenol-free diet (avoiding fruits and vegetables, nuts, legumes, high-fiber products, and beverages such as tea, coffee and fruit juices, as well as alcohol) for 2 days before fecal collection. Samples were immediately stored in an anaerobic jar and then diluted with Dulbecco's phosphate buffer saline at 1% (w/v) and homogenized to obtain a 10% (w/w) slurry to be used as the fermentation starter.

2.4.3 Fermentation conditions

The final fermentation volume was 4 mL, made of 45% growth medium, 45% of fecal slurry, and 10% of food sample extract (prepared dissolving 0.5 g of dried sample in 4 mL of PBS).

The fermentation starter and samples were introduced in the vessel containing sterilized growth medium, sealed with a rubber seal, and flushed through a double needle with nitrogen to create an anaerobic condition. Vessels were then incubated for 24 h at 37°C at 200 strokes/min in a Dubnoff bath (ISCO, Milan, Italy) and collected after 15 min, 5 and 24 h for further analysis. Following incubation, fecal metabolism was stopped by adding 0.4 mL of acetonitrile to the 4 mL of fermented sample and samples were frozen (-18°C) until metabolite extraction and subsequent analysis. All experiments were carried out in triplicate.

2.5 Phenolic compound and metabolite extraction.

The extraction of free (poly)phenolic compounds from raw and cooked both non-digested and digested green pepper was performed according to Sánchez-Salcedo, Mena, Garcia-Viguera, Martinez & Hernandez (2015) with some modifications. Briefly, 50 mg of each sample was extracted with 1 mL of methanol/acidified water (0.1% formic acid) (80:20 v/v), sonicated for 90 min, followed immediately by 1 min of vortex mixing, and centrifuged for 10 min at 14000 rpm (HERMLE Labortechnik GmbH, Wehingen, Germany). The supernatant was collected and the residue was re-extracted using 0.5 mL of methanol/acidified water (80:20 v/v), sonicated for 25 min in a sonic bath, vortex for 1 min and centrifuged for 10 min at 14000 rpm. Both supernatants were combined and stored in the freezer at -18°C until LC/MSⁿ analyses.

The extraction of the bound (poly)phenolic compounds was performed following the method reported by Zaupa et al. (2014). The residue obtained from previous extractions was further hydrolyzed with 1.5 mL of 2 M sodium hydroxide and kept at room temperature for 1 h. After alkaline hydrolysis, the pH of the mixture was adjusted to pH 3 by adding 1.35 mL of 3 M citric acid. The bound phenolic compounds were then extracted with 4 mL of ethyl acetate. After 10 min at 14000 rpm centrifugation, 1 mL of the ethyl acetate supernatant was dried

under vacuum by rotary evaporation (Savant SPD121P, SpeedVac Concentrator, Thermo Scientific, Inc., San Jose, CA, U.S.A.) and the residue was dissolved in 0.25 mL of methanol/acidified water (0.1% formic acid) (80:20 v/v) for the LC/MSⁿ analyses.

For the extraction of fecal metabolites, 0.1 mL aliquots of fermented samples were transferred to a clean microfuge tube. (Poly)phenolic compounds and possible metabolites derived from the fecal fermentation were extracted by adding 0.1 mL of methanol/acidified water (0.1% formic acid) (80:20 v/v), vortexed and centrifuged at 14000 rpm for 10 min. Aliquots of 100 μ L were then transferred to vials and subjected to LC/MSⁿ analysis.

2.6 Identification and quantification of (poly)phenolic compounds and metabolites

Qualitative and quantitative analysis of (poly)phenolic compounds were carried out using an Accela UHPLC 1250 equipped with a linear ion trap mass spectrometer (LTQ XL, Thermo Fisher Scientific Inc., San Jose, CA, U.S.A.) fitted with a heated ESI probe (H-ESI-II, Thermo Fisher Scientific Inc., San Jose, CA, U.S.A.).

To determine (poly)phenols of green pepper, a preliminary analysis was carried out in a full scan, data-dependent MS³ mode, scanning from m/z of 100 to 1500 to identify the compounds. Consequently, a selective full scan MS² mode analysis, monitoring specific m/z , was performed to quantify the previous identified (poly)phenolic compounds. For UHPLC separation, mobile phase A was 0.1% (v/v) formic acid in acetonitrile and mobile phase B was 0.1% (v/v) formic acid in water. Separations were carried out by means of a C18 BlueOrchid column (50 \times 2 mm; 1.8 μ m particle size; Knauer, Berlin, Germany), with an injection volume of 5 μ L, column oven temperature of 30 $^{\circ}$ C and elution flow rate of 0.3 mL/min. The mobile phases comprised a program of 0-3 min, 5% A; 3-12 min, 5-40% A; 12-13 min, 40-80% A; 13-16 min, 80% A and then return to 5% A in one min and maintained the gradient until the end of the analysis (21 min) to re-equilibrate the column. The MS functioned in

negative ionization mode, with capillary temperature set at 275 °C, while the source was maintained at 300 °C. The sheath gas flow was 50 units, while auxiliary and sweep gases were set both to 5. The source voltage was 3 kV and the capillary voltage and tube lens were -2 and -58 V, respectively. All compounds were fragmented with pure helium gas (99.9999%) using a CID of 35.

The analysis of potential phenolic metabolites resulted from the *in vitro* human fecal microbiota degradation, was also performed firstly in full scan, data-dependent MS³ mode, scanning from m/z of 100 to 1500, for a comprehensive compound identification, and, later, in a full scan data-dependent MS² mode, monitoring the specific identified ions for the quantification. Chromatographic separation was performed using a XSELECTED HSS T3 (50 x 2.1 mm, 2.5 µm particle size, Waters, Milford, MA, USA) and employing the conditions and the same solvents previously described, except for the elution gradient. The mobile phase was made of 0-0.5 min, 2% A; 0.5-9 min, 2-45% A; 9-9.5 min, 45-80% A; 9.5-12.5 min, 80% A and then return to 2% A in 0.5 min. The MS worked in negative ionization mode, with a capillary temperature of 275 °C, while the source was maintained at 250 °C. The sheath gas flow was 40 units, while auxiliary and sweep gases were set to 5 units. The source voltage was 3 kV, and the capillary voltage and tube lens were -9 and -53 V, respectively. All metabolites were fragmented using a CID of 30.

Quantification was performed with calibration curves built with the available standard compounds. Quercetin derivatives were quantified in rutin equivalents. Luteolin derivatives were quantified as luteolin 4-glucoside equivalents, all caffeoylquinic acids were quantified using 5-CQA, while caffeic acid derivatives and coumaric acid derivatives were quantified respectively as caffeic acid and coumaric acid equivalents.

Chromatograms and spectral data were acquired using XCalibur software 2.1 (Thermo Fisher Scientific Inc., San Jose, CA, U.S.A.). Each sample was analysed in triplicate.

2.7 Bioaccessibility of (poly)phenolic compounds

The percentage of bioaccessibility of (poly)phenolic compounds after simulated gastrointestinal digestion or fecal fermentation was calculated as following:

$$\text{Bioaccessibility (\%)} = \frac{\text{PCA}}{\text{PCB}} * 100$$

Where PCA is the total (Poly)phenolic Compounds content in samples (nmol/g dm) After *in vitro* digestion or fecal fermentation and PCB is the total (Poly)phenolic Compounds content in samples (nmol/g dm) Before *in vitro* digestion or fecal fermentation.

2.8 Statistical analysis

Results are shown as the mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was applied for each parameter. Bonferroni test was applied as *a posteriori* test for detecting significantly different means ($p < 0.05$). All statistical analyses were performed using the STATA v.12.0 software package.

3. Results and discussion

A total of 21 (poly)phenolic compounds were identified and quantified (Table 1) in green pepper samples. Specifically, 6 quercetin derivatives, 9 luteolin derivatives and 6 hydroxycinnamic acids were detected and quantified. Flavonoids, particularly quercetin rhamnoside, were the main compounds found both in raw and cooked samples. All flavonoids were mainly detected in the analyzed free fraction, although some of them, as rutin, quercetin glucoside, quercetin rhamnoside, luteolin 8-C-hexoside and luteolin 7-O-(2-apiosyl) glucoside, were also found in the bound fraction. In contrast, some hydroxycinnamic

acids, such as caffeic and coumaric acids, were found only in the bound fraction. These compounds could probably be linked to pepper fiber fraction and released from the food matrix only after the hydrolysis process. Additionally, the applied alkaline hydrolysis process could have degraded some phenolic compounds, such as caffeic acids derivatives, CQAs or coumaroylquinic acid, so they could be detected after hydrolysis into their corresponding caffeic and coumaric acids (Monente, Ludwig, Irigoyen, De Pena, & Cid, 2015 a).

Figure 1 and Table 1 show free and bound (poly)phenolic compounds of green pepper which have been quantified by UHPLC both in raw and in cooked vegetables treated with different cooking methods, comparing also how the *in vitro* gastrointestinal digestion affected the final amount of (poly)phenolic compounds. Before digestion, raw pepper presented a total of 12.664 μmol (poly)phenolic compounds/g dm, of which 66% were detected as free compounds and 34% as bound compounds. After submitting green pepper to a frying process both with olive oil and sunflower oil, total (poly)phenolic compounds decreased by more than a half, resulting reduced to 4.715 and 5.113 $\mu\text{mol/g}$ dm in olive oil and sunflower oil fried green pepper, respectively. Although free (poly)phenolic compounds decreased significantly after frying process, the overall loss of (poly)phenolic compounds was mainly due to the decrease of bound compounds. Furthermore, phenolic compounds exclusively found in olive oil, such as oleuropein, pinorelinol, tyrosol and hydroxytyrosol were not detected in olive oil fried green pepper, probably because olive oil used for frying was a blend mainly constituted by refined olive oil with a little amount of virgin olive oil, where phenolic compounds were hardly present (Boskou, 2009). Similarly, sunflower oil was also refined, and (poly)phenols identified in oil fried green pepper samples were the same detected in raw ones. Therefore, the contribution of the oils to the (poly)phenols of cooked samples was scarce.

The amount of (poly)phenolic compounds of griddled green pepper (11.475 $\mu\text{mol/g dm}$) was also lower than in raw green pepper, but the reduction was much lower than after frying process. Free (poly)phenolic compounds were strongly affected, but bound compounds decreased only 5%, representing the 29% of the total (poly)phenolic compounds of griddled green pepper. The decrease of bound compounds observed in all cooked samples with respect to raw green pepper, could be due to the thermal destruction of cell walls and sub-cellular compartments during the cooking process that increases the release of these compounds. The lower release of bound compounds in the griddled samples was probably due to the effect of the higher temperature applied during this cooking process compared to frying, which could increase the formation of high molecular weight end products typical for the Maillard reaction, such as melanoidins, that could retain phenolic compounds into their structures. Some studies confirmed that higher roasting temperature induced higher formation of melanoidins and that melanoidins content also depends on the extent of roasting (Bekedam, Loots, Schols, Van Boekel, & Smit, 2008 a; Sacchetti et al., 2016). Additionally, some studies about coffee reported the incorporation of chlorogenic acids and other phenolic compounds into melanoidins, which may reach an astounding 54% mainly by non-covalent interactions (Bekedam, Schols, Van Boekel, & Smit, 2008 b; Monente et al., 2015 a; Morales, Somoza, & Fogliano, 2012; Nunes & Coimbra, 2010).

Simulated gastrointestinal digestion

The (poly)phenolic compound composition was also influenced by gastrointestinal digestion (Figure 1). In raw green pepper, an evident decrease (more than 50%) of total (poly)phenolic compounds was observed after digestion. The loss was mainly due to the decrease of the bound fraction, which represented the 34% of total (poly)phenolic compounds in raw green pepper before digestion and was reduced to 11% after digestion. This change may be due to

the release of the bound compounds from the food matrix as a consequence of the enzymatic action. In griddled pepper, a decrease in total (poly)phenolic compounds (around 20%) was also observed after gastrointestinal digestion, however the loss was lower than what occurred in raw samples. The high amount of bound compounds, probably attached to complex structures derived from the Maillard reaction which took place during the griddled process, appeared to be more stable during the *in vitro* digestion process, resulting in their release from the food matrix without any subsequent degradation. In fried samples, both with olive oil and sunflower oil, the fat content of the samples could have exerted a protective effect against enzymatic action and the final amount of (poly)phenolic compounds was not affected by gastrointestinal digestion. In olive oil fried green pepper only 5% of total (poly)phenolic compounds were degraded by enzymatic action and no significant changes were observed in the amount of total (poly)phenolic compounds of sunflower oil fried green pepper. Considering the individual (poly)phenolic compounds (Table 1), no new compounds were found after the digestion process although aglyconic forms could be expected. Actually, the loss of the glycosidic moieties is due to membrane-bound glycosylases found on the brush border of the mammalian small intestine (Day et al., 2000; Nemeth et al., 2003), which are clearly not present under the adopted conditions.

Finally, it can be concluded that the bioaccessibility of phenolic compounds after gastrointestinal digestion was higher in cooked samples than in raw ones. The 82%, 96% and 100% of the total amount of (poly)phenolic compounds of undigested griddled green pepper, olive oil fried green pepper and sunflower oil fried green pepper, respectively, were bioaccessible after gastrointestinal digestion, compared to 48% in raw pepper. These results are in accordance with the data reported by others, who demonstrated a higher bioaccessibility of (poly)phenolic compounds after food thermal treatment (Girgin & El Nehir, 2015).

Furthermore, griddled pepper showed the largest amount of phenolic compounds still present in the matrix after the digestion process (9.447 μmol (poly)phenolic compounds/g dm).

(Poly)phenolic compounds degradation during fecal fermentation

After the intake of flavonoids and non-flavonoids, the circulation levels of these compounds in plasma are low (Aura, 2008), probably related to their limited absorption (Dupas, Baglieri, Ordonaud, Tome, & Maillard, 2006; Jaganath, Mullen, Edwards, & Crozier, 2006; Manach et al., 2005; Monente et al., 2015 b; Walle, 2004). Moreover, polyphenol bioavailability in the first gastrointestinal tract has been estimated less than 20% (Hu, 2007). In the present study, the (poly)phenolic compounds detected in digested samples have been considered as the compounds which potentially reach the colon and could be metabolized by the microbiota.

(Poly)phenolic profiles of green pepper at the beginning of the experiment and during the fecal fermentation (15 min, 5 h and 24 h of incubation) are shown in Figure 2. An important microbial metabolic activity was observed, which resulted in the hydrolysis of flavonoid glycosides and the consequent formation of aglycones, principally quercetin and luteolin, as a first step of the fecal fermentation (Figure 2 A). Quercetin derivatives were quickly metabolized and during the first few minutes of colonic biotransformation, the amount of quercetin-based compounds was halved, while quercetin aglycone increased simultaneously. The highest amount of quercetin was detected after 5 hours of fecal incubation. However, the recovered amount of quercetin after 5 h did not correspond to the total of the native quercetin derivatives, indicating that also the aglycone, once released, could be rapidly degraded, in agreement with previously reported results (Serra et al., 2012). Similarly, luteolin aglycone was rapidly released at the beginning of the fecal incubation. However, luteolin derivatives were not degraded so fast, letting hypothesize that luteolin could have also derived from quercetin dehydroxylation, as illustrated in Figure 3. In general, *O*-glucosides of both

quercetin and luteolin were almost completely metabolized by the intestinal microbiota while C-glycosides were much more slowly degraded, and some of them, as for example luteolin 8-C-hexoside, the main flavonoid derivative found in all samples after fecal fermentation (Tables 2, 3, 4 and 5), were still present after 24 h of fecal incubation. This result is in agreement with those reported by Hein, Rose, Van't Slot, Friedrich & Humpf (2008), who observed a complete metabolism of *O*-glycoside compounds between 20 min and 4 hours, whereas C-glycoside compounds which only partially reduced. The same study showed that the released aglycones were completely metabolized within 8 hours. Nevertheless, in the present study, luteolin aglycone was substantially metabolized within 5 hours, whereas low amounts of quercetin still remained after 24 h of fecal incubation. The highlighted difference between the compared studies could be linked to the source of native compounds used in the model, as Hein and colleagues (2008) employed only standard molecules, not a food matrix. Therefore, it could be hypothesized that food matrices used in the present study could have influenced the metabolism of quercetin derivatives, preventing their complete degradation. The catabolic pathways proposed for quercetin and luteolin microbial degradation in the colon are shown in Figure 3. In accordance with Serra et al. (2012), quercetin is subjected to ring fission, resulting in the formation of dihydrocaffeic acid, which could be then further degraded to new catabolites. Ring fission could also result in the formation of protocatechuic acid (Rechner et al., 2004). In the case of luteolin, according to Serra et al. (2012), only dihydrocaffeic acid could be generated.

Concerning phenolic acids, the compounds detected on the digested fraction were quickly metabolized by gut microbiota and no native compounds were detected after 5 h of fecal incubation (Figure 2 B). An increase in caffeic acid amount was detected at the beginning of the fecal fermentation, probably due to the cleavage of quinic acids (Ludwig, de Peña, Cid, &

Crozier, 2013; Rechner et al., 2004; Tomás-Barberán et al., 2014) and to the deglycosilation of caffeic acid glucosides, as illustrated in Figure 3. In agreement with Breynaert et al. (2015), caffeoylquinic acids and their main metabolite, i.e. caffeic acid, were not detectable anymore after 5 h of colonic incubation, indicating a complete degradation of these compounds. In accordance with the literature (Ludwig et al., 2013; Rechner et al., 2004; Tomás-Barberán et al., 2014), caffeic acid was further metabolized into dihydrocaffeic acid by reduction of the double bond.

Additionally to caffeic acid, quercetin and luteolin aglycones, a total of 3 catabolites, namely 3,4-dihydroxybenzoic acid (protocatechuic acid), dihydrocaffeic acid and 3-(3'-hydroxyphenyl)propionic acid were generated during fecal fermentation as products of degradation of (poly)phenolic compounds (Figure 2 C). As previously discussed, dihydrocaffeic acid could be considered an intermediate catabolite of quercetin and caffeic acid catabolism, which undergoes further dihydroxylation and results in the production of 3-(3'-hydroxyphenyl)propionic acid, by far the most abundant catabolite found after fecal incubation of green pepper samples. Moreover, protocatechuic acid was detected in substantial amounts in 5 h fermented samples, and still remained in considerable amounts after 24 h fecal incubation in cooked green pepper (Tables 2, 3, 4 and 5). As previously discussed, it could be generated through the ring fission of quercetin (Rechner et al., 2004), or through the α -oxidation of dihydrocaffeic acid passing via 3',4'-dihydroxyphenylacetic acid (homoprotocatechuic acid) as intermediate (Ludwig et al., 2013). However, dihydroxyphenylacetic acid was not detected in the present study, probably because of its rapid rate of conversion to 3,4-dihydroxybenzoic acid (protocatechuic acid) (Ludwig et al., 2013). Actually, some authors (Aura et al., 2002) detected a higher concentration of dihydroxyphenylacetic acid within 2 h of incubation, a time point not considered in the

present study. Like dihydroxyphenylacetic acid, other minor catabolites previously reported as being generated after (poly)phenol compound fecal biotransformation, such as phenylacetic acid, hydroxyphenylacetic acid, hydroxybenzoic acid or benzoic acid (Aura et al., 2002; Ludwig et al., 2013; Serra et al., 2012) were not detected in the present study. This could be due to the different fermentation times applied, as 3-hydroxyphenylacetic acid was formed by dehydroxylation of 3,4-dihydroxyphenylacetic acid after 8 h incubation (Aura et al., 2002), while other catabolites presented the maximum amount after 48 h of fecal incubation and only low quantities were detected after 24 h (Serra et al., 2012).

Finally, regarding the influence of heat treatment on fecal metabolism, only differences in the total amount of catabolites were observed between raw and cooked green pepper samples (Tables 2, 3, 4 and 5), whereas no differences were detectable in the number of catabolites and in the suggested metabolic pathways for (poly)phenolic microbial degradation in the colon. The total amount of (poly)phenolic compounds and catabolites in green pepper samples remained around 47-59 % bioaccessible after 24 h of fecal incubation. Griddled green pepper, which was the sample with the highest amount of (poly)phenolic compounds after the *in vitro* gastrointestinal digestion, presented the highest amount of compounds after colonic fermentation (4198 $\mu\text{mol/g dm}$), following by sunflower oil fried pepper, raw pepper and olive oil fried pepper (2.719, 2.480 and 2.210 $\mu\text{mol/g dm}$, respectively). No significant differences were found between raw and fried pepper, both with sunflower oil and olive oil.

4. Conclusions

In summary, despite the consistent degradation of (poly)phenolic compounds after cooking processes, the different heat treatments applied in this study seemed to exert a positive effect on the release of phenolic compounds from green pepper during gastrointestinal digestion. In griddled green pepper, the higher temperature applied during this thermal process compared

to frying could increase the formation of Maillard reaction compounds, such as melanoidins, which could form complex structures with (poly)phenolic compounds attached, resulting less accessible to digestive enzyme activities and improving their stability during digestion steps. On the other hand, the fat content of the fried samples could exert a protective effect against enzymatic action. Thus, the bioaccessibility of phenolic compounds after gastrointestinal digestion was higher in cooked samples than in raw one, especially in griddled green pepper, which showed the highest amount of phenolic compounds after the digestion process. Additionally, gut microbiota showed a high metabolic activity resulting in a large modification of (poly)phenolic compounds into new metabolites. Griddled green pepper was still the sample with the highest amount of bioaccessible (poly)phenolic compounds, even after the fecal fermentation step.

The metabolites formed during fecal fermentation may have an influence on the intestinal microflora (Blaut, Schoefer, & Braune, 2003) and their bioactive properties can be different from the activity of their parent compounds. Some studies demonstrated beneficial effects of phenolic catabolites, such as antioxidant, anti-inflammatory, anti-hyperglycemic, neuroprotective activities and positive effects on oxidative stress (Duda-Chodak et al., 2015; Masella et al., 2012; Verzelloni et al., 2011). However, the positive effects of these metabolites on health are still not clearly defined, and more studies are necessary in order to better understand the real actions of these compounds within the human organism. Further studies are also clearly needed to investigate the absorption of (poly)phenolic compounds and their subsequent transformation during phase II enzymatic metabolism resulting in glucuronidated, sulphated and methylated derivatives (Aura, 2008; Del Rio et al., 2013).

Acknowledgments

This research was funded by the Spanish Ministry of Economy and Competitiveness (AGL2014-52636-P). Isabel Juárez wishes to express her gratitude to the Association of Friends of the University of Navarra for the grants received. Iziar Ludwig is supported by a postdoctoral fellowship funded by the Spanish Ministry of Economy and Competitiveness (FJCI-2014-20689).

References

- Aura, A. M., O'Leary, K. A., Williamson, G., Ojala, M., Bailey, M., Puupponen-Pimia, R... Poutanen, K. (2002). Quercetin derivatives are deconjugated and converted to hydroxyphenylacetic acids but not methylated by human fecal flora in vitro. *Journal of Agricultural and Food Chemistry*, 50(6), 1725-1730.
- Aura, A. M. (2008). Microbial metabolism of dietary phenolic compounds in the colon. *Phytochemistry Reviews*, 7(3), 407-429.
- Bekedam, E. K., Loots, M. J., Schols, H. A., Van Boekel, M. A. J. S., & Smit, G. (2008 a). Roasting effects on formation mechanisms of coffee brew melanoidins. *Journal of Agricultural and Food Chemistry*, 56(16), 7138-7145.
- Bekedam, E. K., Schols, H. A., Van Boekel, M. A. J. S., & Smit, G. (2008 b). Incorporation of chlorogenic acids in coffee brew melanoidins. *Journal of Agricultural and Food Chemistry*, 56(6), 2055-2063.
- Blaut, M., Schoefer, L., & Braune, A. (2003). Transformation of flavonoids by intestinal microorganisms. *International Journal for Vitamin and Nutrition Research*, 73(2), 79-87.

- Breynaert, A., Bosscher, D., Kahnt, A., Claeys, M., Cos, P., Pieters, L., & Hermans, N. (2015). Development and validation of an in vitro experimental GastroIntestinal dialysis model with colon phase to study the availability and colonic metabolism of polyphenolic compounds. *Planta Medica*, 81(12-13), 1075-1083.
- Boskou, D. (2009). *Olive oil. Minor constituents and health*. New York, NY: CRC Press.
- Dall'Asta, M., Calani, L., Tedeschi, M., Jechiu, L., Brighenti, F., & Del Rio, D. (2012). Identification of microbial metabolites derived from in vitro fecal fermentation of different polyphenolic food sources. *Nutrition*, 28(2), 197-203.
- Day, A. J., Canada, F. J., Diaz, J. C., Kroon, P. A., Mclauchlan, R., Faulds, C. B., ...Williamson, G. (2000). Dietary flavonoid and isoflavone glycosides are hydrolysed by the lactase site of lactase phlorizin hydrolase. *FEBS Letters*, 468(2-3), 166-170.
- Del Rio, D., Rodriguez-Mateos, A., Spencer, J. P. E., Tognolini, M., Borges, G., & Crozier, A. (2013). Dietary (poly)phenolics in human health: Structures, bioavailability, and evidence of protective effects against chronic diseases. *Antioxidants & Redox Signaling*, 18(14), 1818-1892.
- Duda-Chodak, A., Tarko, T., Satora, P., & Sroka, P. (2015). Interaction of dietary compounds, especially polyphenols, with the intestinal microbiota: A review. *European Journal of Nutrition*, 54(3), 325-341.
- Dupas, C., Baglieri, A. M., Ordonaud, C., Tome, D., & Maillard, M. (2006). Chlorogenic acid is poorly absorbed, independently of the food matrix: A caco-2 cells and rat chronic absorption study. *Molecular Nutrition & Food Research*, 50(11), 1053-1060.

- Eurostat. (2015). Statisticas books. agriculture, forestry and fishery statistics, 2015 edition.
URL <http://ec.europa.eu/eurostat/documents/3217494/7158355/KS-FK-15-101-EN-N.pdf/79470e8c-abf3-43d3-8cd4-84880962cdd4>. Accessed 21.06.16
- Girgin, N., & El Nehir, S. (2015). Effects of cooking on in vitro sinigrin bioaccessibility, total phenols, antioxidant and antimutagenic activity of cauliflower (*Brassica oleraceae* L. Var. Botrytis). *Journal of Food Composition and Analysis*, 37, 119-127.
- Hein, E., Rose, K., Van't Slot, G., Friedrich, A. W., & Humpf, H. (2008). Deconjugation and degradation of flavonol glycosides by pig cecal microbiota characterized by fluorescence in situ hybridization (FISH). *Journal of Agricultural and Food Chemistry*, 56(6), 2281-2290.
- Hu, M. (2007). Commentary: Bioavailability of flavonoids and polyphenols: Call to arms. *Molecular Pharmaceutics*, 4(6), 803-806.
- Jaganath, I. B., Mullen, W., Edwards, C. A., & Crozier, A. (2006). The relative contribution of the small and large intestine to the absorption and metabolism of rutin in man. *Free Radical Research*, 40(10), 1035-1046.
- Juániz, I., Ludwig, I. A., Huarte, E., Pereira-Caro, G., Moreno-Rojas, J. M., Cid, C., & de Peña, M. P. (2016). Influence of heat treatment on antioxidant capacity and (poly)phenolic compounds of selected vegetables. *Food Chemistry*, 197, 466-473.
- Ludwig, I. A., de Peña, M. P., Cid, C., & Crozier, A. (2013). Catabolism of coffee chlorogenic acids by human colonic microbiota. *Biofactors*, 39(6), 623-632.

MAGRAMA (Ministry of Agriculture, Food and Environment) (2015). Informe del consumo de alimentación en España 2015. URL http://www.magrama.gob.es/es/alimentacion/temas/consumo-y-comercializacion-y-distribucion-alimentaria/informeconsumoalimentacion2015_tcm7-422694.pdf. Accessed 21.06.16

Manach, C., Williamson, G., Morand, C., Scalbert, A., & Remesy, C. (2005). Bioavailability and bioefficacy of polyphenols in humans. I. review of 97 bioavailability studies. *American Journal of Clinical Nutrition*, 81(1), 230S-242S.

Marin, A., Ferreres, F., Tomas-Barberan, F. A., & Gil, M. I. (2004). Characterization and quantitation of antioxidant constituents of sweet pepper (*capsicum annum* L.). *Journal of Agricultural and Food Chemistry*, 52(12), 3861-3869.

Masella, R., Santangelo, C., D'Archivio, M., LiVolti, G., Giovannini, C., & Galvano, F. (2012). Protocatechuic acid and human disease prevention: Biological activities and molecular mechanisms. *Current Medicinal Chemistry*, 19(18), 2901-2917.

Miglio, C., Chiavaro, E., Visconti, A., Fogliano, V., & Pellegrini, N. (2008). Effects of different cooking methods on nutritional and physicochemical characteristics of selected vegetables. *Journal of Agricultural and Food Chemistry*, 56(1), 139-147.

Minekus, M., Alming, M., Alvito, P., Ballance, S., Bohn, T., Bourlieu, C., ... Brodkorb, A. (2014). A standardised static in vitro digestion method suitable for food - an international consensus. *Food & Function*, 5(6), 1113-1124.

- Monente, C., Ludwig, I. A., Irigoyen, A., de Peña, M. P., & Cid, C. (2015 a). Assessment of total (free and bound) phenolic compounds in spent coffee extracts. *Journal of Agricultural and Food Chemistry*, 63(17), 4327-4334.
- Monente, C., Ludwig, I. A., Stalmach, A., de Peña, M. P., Cid, C., & Crozier, A. (2015 b). In vitro studies on the stability in the proximal gastrointestinal tract and bioaccessibility in caco-2 cells of chlorogenic acids from spent coffee grounds. *International Journal of Food Sciences and Nutrition*, 66(6), 657-664.
- Morales, F. J., Somoza, V., & Fogliano, V. (2012). Physiological relevance of dietary melanoidins. *Amino Acids*, 42(4), 1097-1109.
- Nemeth, K., Plumb, G. W., Berrin, J. G., Juge, N., Jacob, R., Naim, H. Y., ... Kroon, P. A. (2003). Deglycosylation by small intestinal epithelial cell beta-glucosidases is a critical step in the absorption and metabolism of dietary flavonoid glycosides in humans. *European Journal of Nutrition*, 42(1), 29-42.
- Nunes, F. M., & Coimbra, M. A. (2010). Role of hydroxycinnamates in coffee melanoidin formation. *Phytochemistry Reviews*, 9(1), 171-185.
- Palermo, M., Pellegrini, N., & Fogliano, V. (2014). The effect of cooking on the phytochemical content of vegetables. *Journal of the Science of Food and Agriculture*, 94(6), 1057-1070.
- Pellegrini, N., Miglio, C., Del Rio, D., Salvatore, S., Serafini, M., & Brighenti, F. (2009). Effect of domestic cooking methods on the total antioxidant capacity of vegetables. *International Journal of Food Sciences and Nutrition*, 60(s2), 12-22.

- Ramírez-Anaya, J. P., Samaniego-Sánchez, C., Castañeda-Saucedo, M. C., Villalón-Mir, M., & de la Serrana, H. L. (2015). Phenols and the antioxidant capacity of mediterranean vegetables prepared with extra virgin olive oil using different domestic cooking techniques. *Food Chemistry*, 188(0), 430-438.
- Rechner, A. R., Smith, M. A., Kuhnle, G., Gibson, G. R., Debnam, E. S., Srai, S. K. S., ... Rice-Evans, C. A. (2004). Colonic metabolism of dietary polyphenols: Influence of structure on microbial fermentation products. *Free Radical Biology and Medicine*, 36(2), 212-225.
- Sacchetti, G., Ioannone, F., De Gregorio, M., Di Mattia, C., Serafini, M., & Mastrocola, D. (2016). Non enzymatic browning during cocoa roasting as affected by processing time and temperature. *Journal of Food Engineering*, 169, 44-52.
- Sánchez-Salcedo, E. M., Mena, P., Garcia-Viguera, C., Martinez, J., & Hernandez, F. (2015). Phytochemical evaluation of white (morus alba L.) and black (morus nigra L.) mulberry fruits, a starting point for the assessment of their beneficial properties. *Journal of Functional Foods*, 12, 399-408.
- Scalbert, A., Morand, C., Manach, C., & Remesy, C. (2002). Absorption and metabolism of polyphenols in the gut and impact on health. *Biomedicine & Pharmacotherapy*, 56(6), 276-282.
- Serra, A., Macia, A., Romero, M., Reguant, J., Ortega, N., & Motilva, M. (2012). Metabolic pathways of the colonic metabolism of flavonoids (flavonols, flavones and flavanones) and phenolic acids. *Food Chemistry*, 130(2), 383-393.

- Tomás-Barberán, F., Garcia-Villalba, R., Quartieri, A., Raimondi, S., Amaretti, A., Leonardi, A., & Rossi, M. (2014). In vitro transformation of chlorogenic acid by human gut microbiota. *Molecular Nutrition & Food Research*, 58(5), 1122-1131.
- Verzelloni, E., Pellacani, C., Tagliazucchi, D., Tagliaferri, S., Calani, L., Costa, L.G., ... Del Rio, D. (2011). Antiglycative and neuroprotective activity of colon-derived polyphenol catabolites. *Molecular Nutrition & Food Research*, 55, S35-S43.
- Walle, T. (2004). Absorption and metabolism of flavonoids. *Free Radical Biology and Medicine*, 36(7), 829-837.
- Zaupa, M., Scazzina, F., Dall'Asta, M., Calani, L., Del Rio, D., Bianchi, M. A., ... Brighenti, F. (2014). In vitro bioaccessibility of phenolics and vitamins from durum wheat aleurone fractions. *Journal of Agricultural and Food Chemistry*, 62(7), 1543-1549.

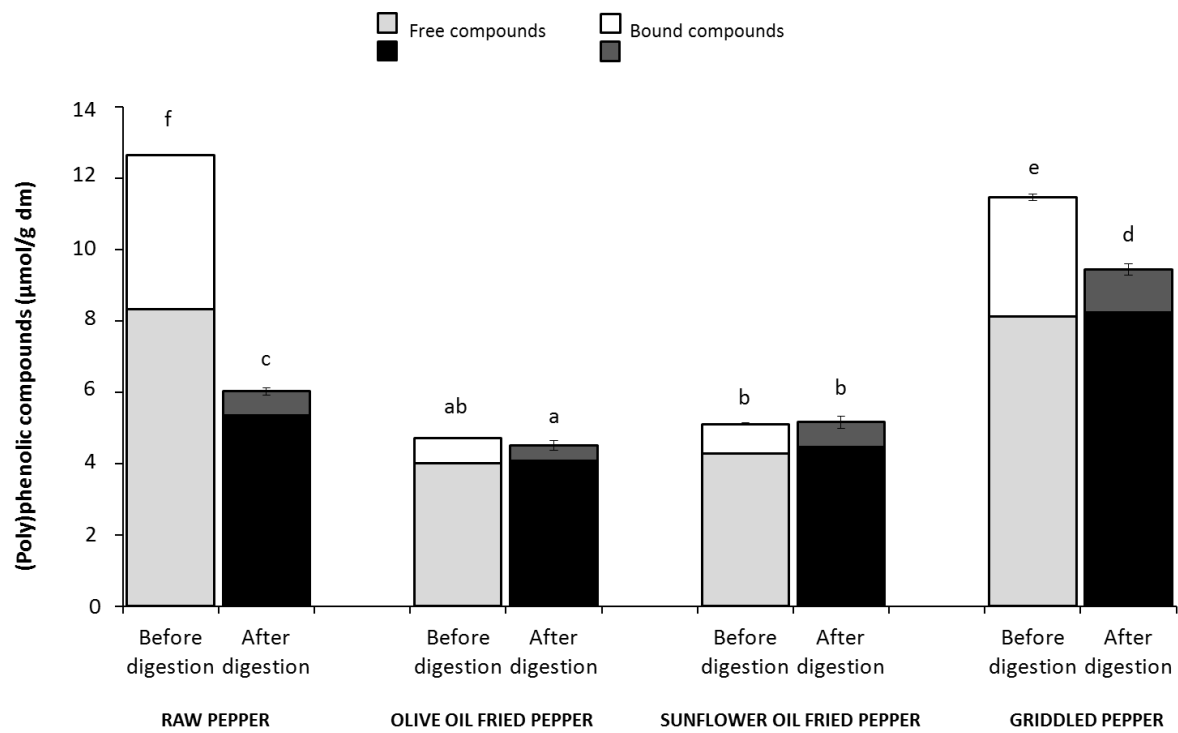
Figure caption

Figure 1. Free, bound and total (poly)phenolic compounds of green pepper both raw and cooked with different heat treatments, and after an *in vitro* gastrointestinal digestion. Results are expressed as mean \pm standard deviation (n=3).

Figure 2. (Poly)phenolic compounds profiles of griddled green pepper during 24 h fecal fermentation. A) Main (poly)phenolic compounds (flavonoids) degradation profiles and production of their corresponding aglycones. B) Minor (poly)phenolic compounds (hydroxycinnamic acids) degradation profiles. C) Main (poly)phenolic catabolites production profiles after *in vitro* fecal fermentation. C is the control sample before fecal fermentation.

Figure 3. Proposed catabolic pathways for microbial degradation of (poly)phenolics in the colon after digestion of green pepper.

Figure 1.



Different letters mean significant differences ($p < 0.05$) between total (poly)phenolic compounds.

Figure 2.

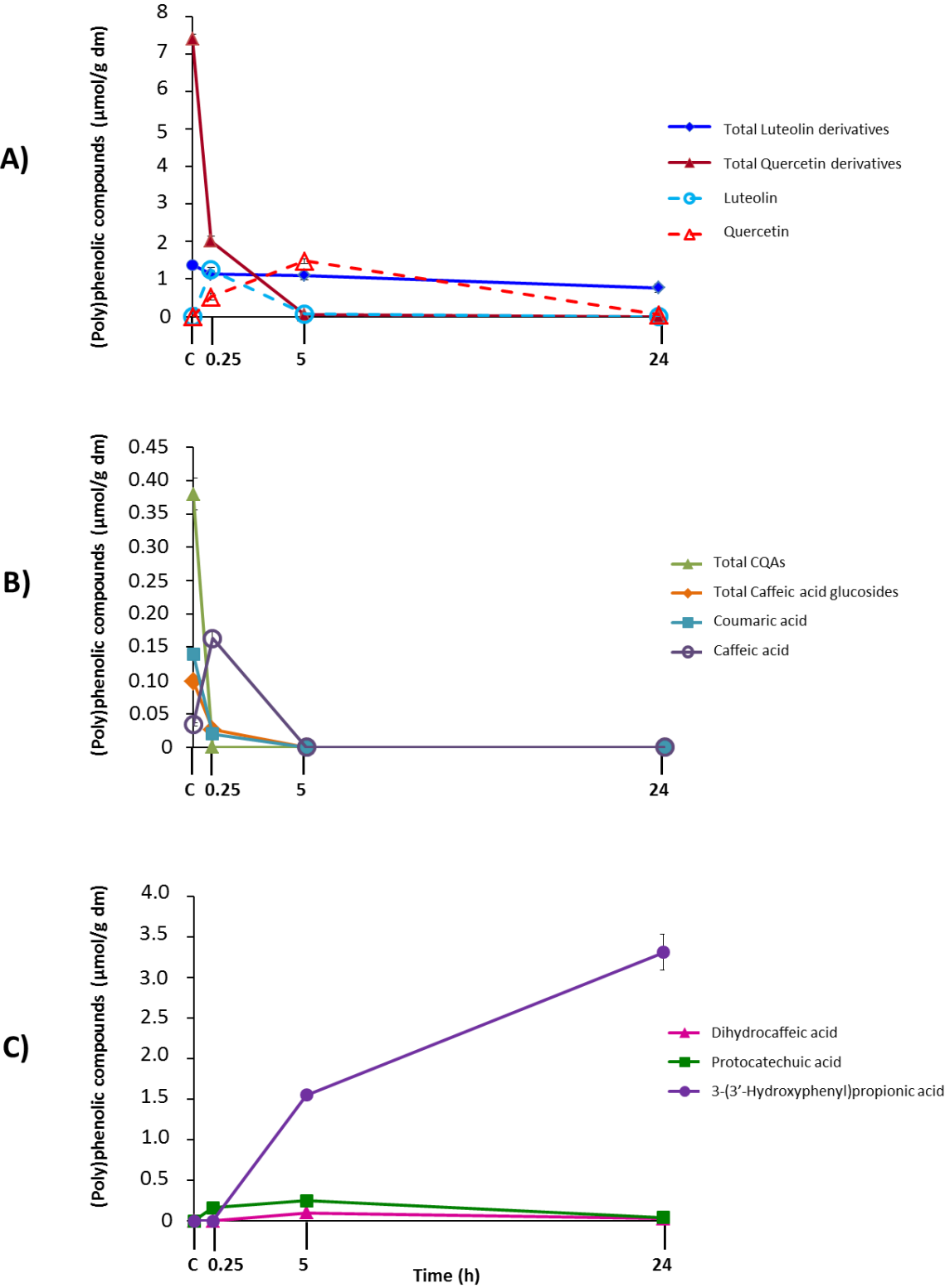
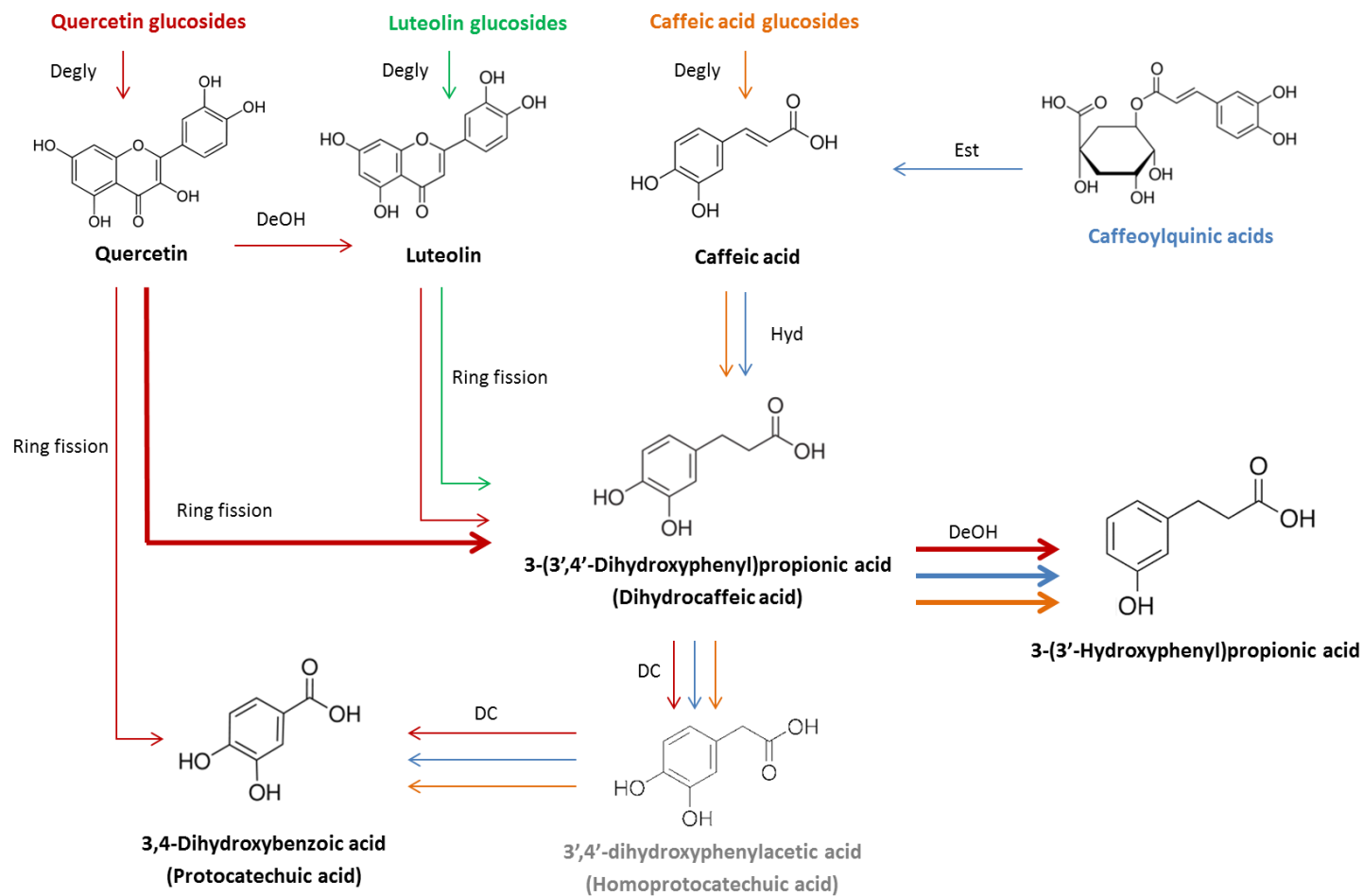


Figure 3.



Degly, Deglycosilation; DeOH, Dehydroxylation; Est, Ester hydrolysis; Hyd, Hydrogenation; DC, Decarboxylation. Detected metabolites are in black, non-detected metabolites are in grey. Red arrows show quercetin glucoside catabolic pathway, green arrows correspond to luteolin derivatives colonic pathways, orange arrows evidence caffeic acid derivatives pathways and blue arrows indicate chlorogenic acid metabolic pathways. Bold arrows indicate major pathways.

Table 1. Free and bound (poly)phenolic compounds in raw and cooked green pepper (fried in olive oil, fried in sunflower oil and griddled) before and after an *in vitro* gastrointestinal digestion. Results are expressed as mean \pm standard deviation (μmol (poly)phenolic compounds/g green pepper dm) (n=3).

Compound	Raw pepper		Olive oil fried pepper		Sunflower oil fried pepper		Griddled pepper	
	Before digestion	After digestion	Before digestion	After digestion	Before digestion	After digestion	Before digestion	After digestion
Quercetin 3-glucoside-7-rhamnoside								
Free compounds	0.043 \pm 0.002 ^c	0.021 \pm 0.003 ^a	0.020 \pm 0.002 ^a	0.019 \pm 0.001 ^a	0.026 \pm 0.003 ^{ab}	0.026 \pm 0.004 ^{ab}	0.039 \pm 0.004 ^{bc}	0.044 \pm 0.005 ^c
Bound compounds	nd	nd	nd	nd	nd	nd	nd	nd
Quercetin 3-sambubioside-7-rhamnoside								
Free compounds	0.096 \pm 0.013 ^c	0.057 \pm 0.000 ^{ab}	0.044 \pm 0.003 ^a	0.038 \pm 0.004 ^a	0.049 \pm 0.004 ^a	0.041 \pm 0.001 ^a	0.084 \pm 0.011 ^{bc}	0.086 \pm 0.003 ^{bc}
Bound compounds	nd	nd	nd	nd	nd	nd	nd	nd
Rutin								
Free compounds	0.073 \pm 0.026 ^{ab}	0.050 \pm 0.007 ^a	0.063 \pm 0.003 ^{ab}	0.062 \pm 0.002 ^{ab}	0.081 \pm 0.010 ^b	0.066 \pm 0.001 ^{ab}	0.178 \pm 0.011 ^d	0.142 \pm 0.001 ^c
Bound compounds	0.003 \pm 0.001 ^b	nd ^a	nd ^a	nd ^a	0.002 \pm 0.000 ^b	0.003 \pm 0.000 ^b	0.007 \pm 0.001 ^c	0.002 \pm 0.000 ^b
Quercetin glucoside								
Free compounds	0.501 \pm 0.116 ^c	0.276 \pm 0.004 ^{ab}	0.238 \pm 0.001 ^{ab}	0.224 \pm 0.008 ^a	0.381 \pm 0.003 ^{bc}	0.331 \pm 0.028 ^{ab}	0.761 \pm 0.085 ^d	0.502 \pm 0.005 ^c
Bound compounds	0.180 \pm 0.003 ^c	0.042 \pm 0.000 ^a	0.031 \pm 0.015 ^a	0.032 \pm 0.003 ^a	0.049 \pm 0.009 ^{ab}	0.060 \pm 0.002 ^{ab}	0.244 \pm 0.013 ^d	0.089 \pm 0.016 ^b
Rutin isomer								
Free compounds	nd ^a	nd ^a	0.010 \pm 0.002 ^b	0.011 \pm 0.002 ^b	0.013 \pm 0.001 ^b	0.011 \pm 0.002 ^b	0.024 \pm 0.001 ^c	0.026 \pm 0.003 ^c
Bound compounds	nd ^a	nd ^a	nd ^a	nd ^a	nd ^a	nd ^a	0.002 \pm 0.000 ^b	nd ^a
Quercetin rhamnoside								
Free compounds	5.001 \pm 0.116 ^d	4.061 \pm 0.032 ^c	2.362 \pm 0.032 ^a	2.820 \pm 0.137 ^{ab}	2.489 \pm 0.022 ^a	3.051 \pm 0.127 ^b	4.797 \pm 0.109 ^d	5.777 \pm 0.214 ^e
Bound compounds	2.911 \pm 0.267 ^d	0.458 \pm 0.008 ^{ab}	0.378 \pm 0.003 ^{ab}	0.243 \pm 0.036 ^a	0.487 \pm 0.016 ^{ab}	0.404 \pm 0.040 ^{ab}	2.020 \pm 0.011 ^c	0.753 \pm 0.081 ^b
Luteolin 6,8-di-C-glucoside								
Free compounds	0.040 \pm 0.000 ^{ab}	0.026 \pm 0.001 ^{ab}	0.035 \pm 0.001 ^{ab}	0.026 \pm 0.000 ^{ab}	0.043 \pm 0.004 ^b	0.024 \pm 0.000 ^a	0.061 \pm 0.001 ^c	0.063 \pm 0.010 ^c
Bound compounds	nd	nd	nd	nd	nd	nd	nd	nd
Luteolin 6-C-hexoside-8-C-pentoside								
Free compounds	0.084 \pm 0.003 ^c	0.048 \pm 0.004 ^a	0.066 \pm 0.004 ^b	0.048 \pm 0.006 ^a	0.083 \pm 0.002 ^c	0.044 \pm 0.002 ^a	0.114 \pm 0.001 ^d	0.115 \pm 0.003 ^d
Bound compounds	nd	nd	nd	nd	nd	nd	nd	nd
Luteolin 6-C-pentoside-8-C-hexoside								
Free compounds	0.045 \pm 0.001 ^b	0.026 \pm 0.001 ^a	0.042 \pm 0.000 ^b	0.030 \pm 0.001 ^a	0.044 \pm 0.006 ^b	0.021 \pm 0.002 ^a	0.052 \pm 0.000 ^b	0.066 \pm 0.004 ^c
Bound compounds	nd	nd	nd	nd	nd	nd	nd	nd
Luteolin 8-C-hexoside								
Free compounds	0.281 \pm 0.020 ^d	0.069 \pm 0.001 ^a	0.223 \pm 0.004 ^{cd}	0.151 \pm 0.028 ^b	0.237 \pm 0.006 ^{cd}	0.171 \pm 0.023 ^{bc}	0.363 \pm 0.011 ^e	0.292 \pm 0.003 ^{de}
Bound compounds	0.060 \pm 0.002 ^c	nd ^a	nd ^a	nd ^a	nd ^a	nd ^a	0.040 \pm 0.000 ^b	nd ^a
Luteolin 7-O-(2-apiosyl)glucoside								
Free compounds	0.388 \pm 0.025 ^{ab}	0.573 \pm 0.060 ^c	0.252 \pm 0.025 ^a	0.291 \pm 0.052 ^{ab}	0.306 \pm 0.009 ^{ab}	0.367 \pm 0.008 ^{ab}	0.350 \pm 0.004 ^{ab}	0.435 \pm 0.003 ^{bc}
Bound compounds	0.514 \pm 0.011 ^d	0.099 \pm 0.004 ^a	0.099 \pm 0.017 ^a	0.082 \pm 0.001 ^a	0.099 \pm 0.001 ^a	0.125 \pm 0.014 ^a	0.351 \pm 0.015 ^c	0.181 \pm 0.007 ^b
Luteolin 7-O-(2-apiosyl-6-malonyl)glucoside I								
Free compounds	0.358 \pm 0.020 ^c	0.070 \pm 0.016 ^a	0.124 \pm 0.015 ^b	0.044 \pm 0.011 ^a	0.123 \pm 0.020 ^b	0.059 \pm 0.006 ^a	0.289 \pm 0.012 ^c	0.160 \pm 0.036 ^b
Bound compounds	nd	nd	nd	nd	nd	nd	nd	nd

Luteolin acetylglucoside I								
Free compounds	0.030 ± 0.000 ^c	0.009 ± 0.002 ^a	0.013 ± 0.001 ^a	0.008 ± 0.000 ^a	0.014 ± 0.001 ^a	0.007 ± 0.000 ^a	0.028 ± 0.002 ^{bc}	0.022 ± 0.002 ^b
Bound compounds	nd	nd	nd	nd	nd	nd	nd	nd
Luteolin 7-O-(2-apiosyl-6-malonyl)glucoside II								
Free compounds	nd ^a	nd ^a	0.028 ± 0.005 ^c	0.012 ± 0.000 ^{ab}	0.019 ± 0.007 ^{bc}	0.005 ± 0.001 ^{ab}	0.084 ± 0.002 ^d	0.033 ± 0.002 ^c
Bound compounds	nd	nd	nd	nd	nd	nd	nd	nd
Luteolin acetylglucoside II								
Free compounds	nd ^a	nd ^a	0.002 ± 0.000 ^b	nd ^a	0.003 ± 0.001 ^b	nd ^a	0.020 ± 0.000 ^d	0.010 ± 0.001 ^c
Bound compounds	nd	nd	nd	nd	nd	nd	nd	nd
Caffeic acid glucoside I								
Free compounds	0.183 ± 0.010 ^d	0.068 ± 0.004 ^b	0.142 ± 0.002 ^c	0.072 ± 0.007 ^b	0.124 ± 0.003 ^c	0.032 ± 0.003 ^a	0.243 ± 0.003 ^e	0.062 ± 0.001 ^b
Bound compounds	nd	nd	nd	nd	nd	nd	nd	nd
Caffeic acid glucoside II								
Free compounds	0.918 ± 0.004 ^d	nd ^a	0.026 ± 0.001 ^b	nd ^a	0.026 ± 0.002 ^b	0.026 ± 0.001 ^b	0.043 ± 0.001 ^c	0.037 ± 0.005 ^{bc}
Bound compounds	nd	nd	nd	nd	nd	nd	nd	nd
Caffeic acid								
Free compounds	nd	nd	nd	nd	nd	nd	nd	nd
Bound compounds	0.272 ± 0.010 ^d	nd ^a	0.043 ± 0.000 ^b	nd ^a	0.034 ± 0.007 ^b	0.018 ± 0.003 ^{ab}	0.221 ± 0.013 ^c	0.034 ± 0.002 ^b
5-CQA								
Free compounds	0.967 ± 0.036 [†]	nd ^a	0.280 ± 0.005 ^{cd}	0.209 ± 0.007 ^{bc}	0.201 ± 0.011 ^{bc}	0.156 ± 0.009 ^b	0.517 ± 0.013 ^e	0.335 ± 0.029 ^d
Bound compounds	nd	nd	nd	nd	nd	nd	nd	nd
4-CQA								
Free compounds	0.155 ± 0.0020 ^e	nd ^a	0.046 ± 0.003 ^c	0.023 ± 0.001 ^b	0.024 ± 0.006 ^b	0.028 ± 0.004 ^b	0.085 ± 0.002 ^d	0.045 ± 0.005 ^c
Bound compounds	nd	nd	nd	nd	nd	nd	nd	nd
Coumaric acid								
Free compounds	nd	nd	nd	nd	nd	nd	nd	nd
Bound compounds	0.388 ± 0.008 ^c	0.079 ± 0.009 ^a	0.149 ± 0.002 ^b	0.069 ± 0.006 ^a	0.157 ± 0.005 ^b	0.087 ± 0.005 ^a	0.460 ± 0.022 ^d	0.139 ± 0.002 ^b
Total (poly)phenolic compounds								
Free compounds	8.337 ^c	5.354 ^b	4.016 ^a	4.089 ^a	4.285 ^a	4.464 ^a	8.130 ^c	8.250 ^c
Bound compounds	4.328 ^d	0.679 ^a	0.700 ^a	0.426 ^a	0.828 ^{ab}	0.696 ^a	3.345 ^c	1.198 ^b
Total compounds	12.664[†]	6.033^c	4.715^{ab}	4.514^a	5.113^b	5.150^b	11.475^e	9.447^d

Different letters for each row indicate significant differences ($p \leq 0.05$) among samples.

Table 2. Native (poly)phenolic compounds and catabolites produced during fecal fermentation of raw green pepper. Results are expressed as mean ± standard deviation (μmol (poly)phenolic compounds/g green pepper dm) (n=3).

Compound	Control	15 min	5 h	24 h
Quercetin derivatives				
Quercetin 3-glucoside-7-rhamnoside	0.021 ± 0.003	0.016 ± 0.001	nd	nd
Quercetin 3-sambubioside-7-rhamnoside	0.057 ± 0.000	0.012 ± 0.003	nd	nd
Rutin	0.050 ± 0.007	0.007 ± 0.002	nd	nd
Quercetin glucoside	0.318 ± 0.003	nd	nd	nd
Rutin isomer	nd	nd	nd	nd
Quercetin rhamnoside	4.519 ± 0.024	2.314 ± 0.128	0.029 ± 0.002	nd
Total quercetin derivatives	4.965 ± 0.025	2.348 ± 0.136	0.029 ± 0.002	nd
Quercetin	nd	0.245 ± 0.023	0.619 ± 0.064	0.029 ± 0.008
Luteolin derivatives				
Luteolin 6,8-di-C-glucoside	0.026 ± 0.001	0.046 ± 0.008	0.019 ± 0.001	0.025 ± 0.002
Luteolin 6-C-hexoside-8-C-pentoside	0.048 ± 0.004	0.090 ± 0.003	0.059 ± 0.027	0.049 ± 0.006
Luteolin 6-C-pentoside-8-C-hexoside	0.026 ± 0.001	0.051 ± 0.001	0.037 ± 0.003	0.027 ± 0.005
Luteolin 8-C-hexoside	0.069 ± 0.001	0.150 ± 0.025	0.099 ± 0.02	0.117 ± 0.006
Luteolin 7-O-(2-apiosyl)glucoside	0.672 ± 0.057	0.415 ± 0.024	0.005 ± 0.002	0.001 ± 0.000
Luteolin 7-O-(2-apiosyl-6-malonyl)glucoside I	0.070 ± 0.016	nd	nd	nd
Luteolin acetylglucoside I	0.009 ± 0.002	nd	nd	nd
Luteolin 7-O-(2-apiosyl-6-malonyl)glucoside II	nd	nd	nd	nd
Luteolin acetylglucoside II	nd	nd	nd	nd
Total luteolin derivatives	0.921 ± 0.072	0.753 ± 0.053	0.220 ± 0.035	0.219 ± 0.009
Luteolin	nd	1.220 ± 0.102	0.043 ± 0.010	nd
Hydroxycinnamic acids				
Caffeic acid glucoside I	0.068 ± 0.004	0.049 ± 0.00	nd	nd
Caffeic acid glucoside II	nd	nd	nd	nd
Caffeic acid	nd	0.015 ± 0.002	nd	nd
5-CQA	nd	nd	nd	nd
4-CQA	nd	nd	nd	nd
Coumaric acid	0.079 ± 0.009	0.057 ± 0.014	nd	nd
Catabolites				
Dihydrocaffeic acid	nd	nd	0.047 ± 0.002	nd
Protocatechuic acid	nd	0.235 ± 0.009	0.299 ± 0.006	nd
3-(3'-Hydroxyphenyl)propionic acid	nd	nd	1.213 ± 0.108	2.232 ± 0.204
Total (poly)phenolic compounds	6.033	4.922	2.469	2.480

Table 3. Native (poly)phenolic compounds and catabolites produced during fecal fermentation of olive oil fried green pepper. Results are expressed as mean ± standard deviation (μmol (poly)phenolic compounds/g green pepper dm) (n=3).

Compound	Control	15 min	5 h	24 h
Quercetin derivatives				
Quercetin 3-glucoside-7-rhamnoside	0.019 ± 0.001	0.012 ± 0.002	nd	nd
Quercetin 3-sambubioside-7-rhamnoside	0.038 ± 0.004	0.003 ± 0.000	nd	nd
Rutin	0.062 ± 0.002	nd	nd	nd
Quercetin glucoside	0.256 ± 0.006	nd	nd	nd
Rutin isomer	0.011 ± 0.002	0.012 ± 0.001	nd	nd
Quercetin rhamnoside	3.063 ± 0.101	0.841 ± 0.063	nd	nd
Total quercetin derivatives	3.449 ± 0.107	0.868 ± 0.062	nd	nd
Quercetin	nd	0.608 ± 0.070	0.643 ± 0.036	0.020 ± 0.001
Luteolin derivatives				
Luteolin 6,8-di-C-glucoside	0.026 ± 0.000	0.039 ± 0.006	0.035 ± 0.008	0.025 ± 0.003
Luteolin 6-C-hexoside-8-C-pentoside	0.048 ± 0.006	0.082 ± 0.023	0.058 ± 0.002	0.050 ± 0.001
Luteolin 6-C-pentoside-8-C-hexoside	0.030 ± 0.001	0.050 ± 0.012	0.042 ± 0.001	0.028 ± 0.002
Luteolin 8-C-hexoside	0.151 ± 0.028	0.304 ± 0.030	0.363 ± 0.022	0.361 ± 0.043
Luteolin 7-O-(2-apiosyl)glucoside	0.373 ± 0.054	0.141 ± 0.018	0.004 ± 0.001	nd
Luteolin 7-O-(2-apiosyl-6-malonyl)glucoside I	0.044 ± 0.011	nd	nd	nd
Luteolin acetylglucoside I	0.008 ± 0.000	nd	nd	nd
Luteolin 7-O-(2-apiosyl-6-malonyl)glucoside II	0.012 ± 0.000	nd	nd	nd
Luteolin acetylglucoside II	nd	nd	nd	nd
Total luteolin derivatives	0.693 ± 0.033	0.616 ± 0.088	0.502 ± 0.034	0.464 ± 0.045
Luteolin	nd	0.735 ± 0.088	0.057 ± 0.002	nd
Hydroxycinnamic acids				
Caffeic acid glucoside I	0.072 ± 0.007	0.014 ± 0.002	nd	nd
Caffeic acid glucoside II	nd	nd	nd	nd
Caffeic acid	nd	0.134 ± 0.002	nd	nd
5-CQA	0.209 ± 0.007	nd	nd	nd
4-CQA	0.023 ± 0.001	nd	nd	nd
Coumaric acid	0.069 ± 0.006	0.003 ± 0.000	nd	nd
Catabolites				
Dihydrocaffeic acid	nd	nd	0.057 ± 0.005	nd
Protocatechuic acid	nd	0.131 ± 0.00	0.212 ± 0.000	0.073 ± 0.009
3-(3'-Hydroxyphenyl)propionic acid	nd	nd	0.810 ± 0.102	1.652 ± 0.111
Total (poly)phenolic compounds	4.514	3.109	2.280	2.210

Table 4. Native (poly)phenolic compounds and catabolites produced during fecal fermentation of sunflower oil fried green pepper. Results are expressed as mean ± standard deviation (μmol (poly)phenolic compounds/g green pepper dm) (n=3).

Compound	Control	15 min	5 h	24 h
Quercetin derivatives				
Quercetin 3-glucoside-7-rhamnoside	0.026 ± 0.004	0.015 ± 0.002	nd	nd
Quercetin 3-sambubioside-7-rhamnoside	0.041 ± 0.001	0.006 ± 0.000	nd	nd
Rutin	0.069 ± 0.002	nd	nd	nd
Quercetin glucoside	0.391 ± 0.026	nd	nd	nd
Rutin isomer	0.011 ± 0.002	0.019 ± 0.00	nd	nd
Quercetin rhamnoside	3.455 ± 0.167	1.278 ± 0.192	nd	nd
Total quercetin derivatives	3.991 ± 0.138	1.318 ± 0.197	nd	nd
Quercetin	nd	0.384 ± 0.098	0.435 ± 0.004	0.005 ± 0.000
Luteolin derivatives				
Luteolin 6,8-di-C-glucoside	0.024 ± 0.000	0.056 ± 0.007	0.029 ± 0.007	0.026 ± 0.002
Luteolin 6-C-hexoside-8-C-pentoside	0.044 ± 0.002	0.085 ± 0.003	0.058 ± 0.012	0.064 ± 0.002
Luteolin 6-C-pentoside-8-C-hexoside	0.021 ± 0.002	0.088 ± 0.005	0.049 ± 0.009	0.038 ± 0.005
Luteolin 8-C-hexoside	0.171 ± 0.023	0.424 ± 0.074	0.365 ± 0.054	0.317 ± 0.063
Luteolin 7-O-(2-apiosyl)glucoside	0.492 ± 0.023	0.180 ± 0.021	0.004 ± 0.001	nd
Luteolin 7-O-(2-apiosyl-6-malonyl)glucoside I	0.059 ± 0.005	nd	nd	nd
Luteolin acetylglucoside I	0.007 ± 0.000	nd	nd	nd
Luteolin 7-O-(2-apiosyl-6-malonyl)glucoside II	0.005 ± 0.001	nd	nd	nd
Luteolin acetylglucoside II	nd	nd	nd	nd
Total luteolin derivatives	0.824 ± 0.009	0.833 ± 0.109	0.505 ± 0.080	0.444 ± 0.068
Luteolin	nd	0.913 ± 0.070	0.034 ± 0.008	nd
Hydroxycinnamic acids				
Caffeic acid glucoside I	0.032 ± 0.003	0.019 ± 0.001	nd	nd
Caffeic acid glucoside II	0.026 ± 0.001	nd	nd	nd
Caffeic acid	0.018 ± 0.003	0.135 ± 0.009	nd	nd
5-CQA	0.156 ± 0.009	nd	nd	nd
4-CQA	0.028 ± 0.004	nd	nd	nd
Coumaric acid	0.087 ± 0.005	0.006 ± 0.000	nd	nd
Catabolites				
Dihydrocaffeic acid	nd	nd	0.055 ± 0.002	nd
Protocatechuic acid	nd	0.140 ± 0.003	0.146 ± 0.006	0.034 ± 0.004
3-(3'-Hydroxyphenyl)propionic acid	nd	nd	1.281 ± 0.042	2.236 ± 0.070
Total (poly)phenolic compounds	5.161	3.749	2.457	2.719

Table 5. Native (poly)phenolic compounds and catabolites produced during fecal fermentation of griddled green pepper. Results are expressed as mean ± standard deviation (μmol (poly)phenolic compounds/g green pepper dm) (n=3).

Compound	Control	15 min	5 h	24 h
Quercetin derivatives				
Quercetin 3-glucoside-7-rhamnoside	0.044 ± 0.005	0.019 ± 0.001	nd	nd
Quercetin 3-sambubioside-7-rhamnoside	0.086 ± 0.003	0.005 ± 0.000	nd	nd
Rutin	0.144 ± 0.001	nd	nd	nd
Quercetin glucoside	0.591 ± 0.011	nd	nd	nd
Rutin isomer	0.026 ± 0.003	0.024 ± 0.001	nd	nd
Quercetin rhamnoside	6.530 ± 0.133	1.969 ± 0.126	0.045 ± 0.004	nd
Total quercetin derivatives	7.419 ± 0.116	2.017 ± 0.129	0.045 ± 0.004	nd
Quercetin	nd	0.528 ± 0.094	1.482 ± 0.061	0.052 ± 0.007
Luteolin derivatives				
Luteolin 6,8-di-C-glucoside	0.063 ± 0.010	0.094 ± 0.019	0.073 ± 0.009	0.050 ± 0.006
Luteolin 6-C-hexoside-8-C-pentoside	0.115 ± 0.003	0.159 ± 0.006	0.149 ± 0.012	0.125 ± 0.010
Luteolin 6-C-pentoside-8-C-hexoside	0.066 ± 0.004	0.110 ± 0.021	0.132 ± 0.012	0.060 ± 0.002
Luteolin 8-C-hexoside	0.292 ± 0.003	0.571 ± 0.103	0.733 ± 0.091	0.529 ± 0.098
Luteolin 7-O-(2-apiosyl)glucoside	0.616 ± 0.023	0.197 ± 0.021	0.008 ± 0.001	0.002 ± 0.000
Luteolin 7-O-(2-apiosyl-6-malonyl)glucoside I	0.160 ± 0.036	nd	nd	nd
Luteolin acetylglucoside I	0.022 ± 0.002	nd	nd	nd
Luteolin 7-O-(2-apiosyl-6-malonyl)glucoside II	0.033 ± 0.002	nd	nd	nd
Luteolin acetylglucoside II	0.010 ± 0.001	nd	nd	nd
Total luteolin derivatives	1.377 ± 0.028	1.130 ± 0.078	1.095 ± 0.124	0.767 ± 0.116
Luteolin	nd	1.243 ± 0.063	0.072 ± 0.012	nd
Hydroxycinnamic acids				
Caffeic acid glucoside I	0.062 ± 0.001	0.026 ± 0.001	nd	nd
Caffeic acid glucoside II	0.037 ± 0.005	nd	nd	nd
Caffeic acid	0.034 ± 0.002	0.163 ± 0.011	nd	nd
5-CQA	0.334 ± 0.029	nd	nd	nd
4-CQA	0.045 ± 0.005	nd	nd	nd
Coumaric acid	0.139 ± 0.002	0.020 ± 0.004	nd	nd
Catabolites				
Dihydrocaffeic acid	nd	nd	0.100 ± 0.004	0.027 ± 0.005
Protocatechuic acid	nd	0.162 ± 0.000	0.251 ± 0.001	0.041 ± 0.004
3-(3'-Hydroxyphenyl)propionic acid	nd	nd	1.551 ± 0.055	3.311 ± 0.221
Total (poly)phenolic compounds	9.447	5.289	4.597	4.198